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Mechanisms of IVIG efficacy in chronic inflammatory demyelinating polyneuropathy

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Abstract: **BACKGROUND:** Chronic inflammatory demyelinating polyneuropathy (CIDP) is the most common treatable acquired chronic polyneuropathy. Corticosteroids, plasmapheresis and intravenous immunoglobulins (IVIG) have been shown to be effective in randomized controlled clinical trials and IVIG is widely used as a first-line initial and maintenance treatment for CIDP. Studies in animal models of autoimmune diseases indicated that the inhibitory Fc-gamma receptor FcgammaRIIB, expressed on myeloid cells and B cells, is required for the anti-inflammatory activity of IVIG. **SUMMARY:** We found that untreated patients with CIDP, compared to demographically matched healthy controls, show lower FcgammaRIIB expression levels on naïve B cells and fail to upregulate or to maintain upregulation of FcgammaRIIB as B cells progress from the naive to the memory compartment. Furthermore, FcgammaRIIB protein expression is upregulated on B cells and monocytes following clinically effective IVIG therapy suggesting that impaired expression of the inhibitory FcgammaR in CIDP can, at least partially, be restored by IVIG treatment. In B cells, FcgammaRIIB transduces an inhibitory signal upon colligation with the B cell receptor, thereby preventing B cells with low affinity or self-reactive receptors from entering the germinal center and becoming IgG positive plasma cells. Our data suggest that this late B cell differentiation checkpoint is impaired in CIDP. Modulating FcgammaRIIB function might be a promising approach to efficiently limit antibody-mediated immunopathology in CIDP.

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Mechanisms of IVIG Efficacy in Chronic Inflammatory Demyelinating Polyneuropathy

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Abstract

Background Chronic inflammatory demyelinating polyneuropathy (CIDP) is the most common treatable acquired chronic polyneuropathy. Corticosteroids, plasmapheresis and intravenous immunoglobulins (IVIG) have been shown to be effective in randomized controlled clinical trials and IVIG is widely used as a first-line initial and maintenance treatment for CIDP. Studies in animal models of autoimmune diseases indicated that the inhibitory Fc-gamma receptor FcγRIIB, expressed on myeloid cells and B cells, is required for the anti-inflammatory activity of IVIG.

Summary We found that untreated patients with CIDP, compared to demographically matched healthy controls, show lower FcγRIIB expression levels on naïve B cells and fail to upregulate or to maintain upregulation of FcγRIIB as B cells progress from the naïve to the memory compartment. Furthermore, FcγRIIB protein expression is upregu-

lated on B cells and monocytes following clinically effective IVIG therapy suggesting that impaired expression of the inhibitory FcγR in CIDP can, at least partially, be restored by IVIG treatment. In B cells, FcγRIIB transduces an inhibitory signal upon colligation with the B cell receptor, thereby preventing B cells with low affinity or self-reactive receptors from entering the germinal center and becoming IgG positive plasma cells. Our data suggest that this late B cell differentiation checkpoint is impaired in CIDP. Modulating FcγRIIB function might be a promising approach to efficiently limit antibody-mediated immunopathology in CIDP.

Keywords Chronic inflammatory demyelinating polyneuropathy · CIDP · intravenous immunoglobulin · IVIG

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CIDP: Pathophysiological Aspects

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an acquired immune-mediated disease of the peripheral nervous system. It is the most common form of a heterogeneous group of immune-mediated and, in many cases, well-treatable peripheral neuropathies [1]. The estimated prevalence rate of CIDP differs between 1.61 and 8.9 per 100,000 [2, 3]. Although CIDP occurs at any age, the peak incidence is between 40 and 60 years. The most common form of CIDP causes symmetrical progressive or relapsing weakness that affects proximal and distal muscles with concomitant distal sensory involvement and areflexia [4, 5].

Several mechanisms such as inflammation, demyelination, axonal damage, and repair mechanisms contribute sequentially or simultaneously to the pathophysiology of

CIDP. The disease is believed to be triggered and maintained by an immune response against yet unidentified autoantigens within the peripheral nervous system. In experimental *in vivo* models for autoimmune neuropathies, such as experimental autoimmune neuritis (EAN), the genetic background of the animal will determine the choice of induction of immune response. Immunization with entire myelin homogenates, the major myelin adhesion molecule P0, the fatty acid binding protein P2, myelin basic protein, or with gangliosides that are prevalent in axonal membranes leads to the development of demyelinating or axonal inflammatory neuropathies. Injection of myelin-reactive T cells is sufficient to induce adoptive-transfer EAN in susceptible hosts [6].

Proinflammatory T helper cell type 1 (Th1) signature cytokines such as the chemokine receptor CXCR-3, interferon- γ inducible protein-10, and monokine induced by interferon- γ are detectable in sural nerve biopsies from patients with CIDP and other autoimmune neuropathies [7]. This suggests that, similar to other autoimmune diseases, Th1 cells orchestrate and maintain peripheral nerve damage in CIDP.

Humoral immune responses are thought to play a crucial role in mediating peripheral nerve damage and represent important pharmacological targets in patients with CIDP. Sera and immunoglobulin G (IgG) antibodies from patients induce peripheral demyelination in host animals [8] and can increase the permeability of the blood–nerve barrier and impair nerve conduction in various models of peripheral neuropathies [6]. Removal of humoral immune mediators by plasma exchange therapy as well as through intravenous immunoglobulin (IVIG) is considered first-line treatments in patients with CIDP [9, 10].

Efficacy of IVIG in CIDP: Evidence from Clinical Trials

Corticosteroids, plasmapheresis, and IVIG have been shown to be effective in short-term prospective randomized controlled trials (RCTs). Rituximab, alemtuzumab, etanercept, interferon- β , interferon- α , mycophenolate mofetil, or stem cell transplants were efficacious in individual cases. Plasma-exchange therapy and cyclophosphamide are usually reserved for rapid progressive courses [1].

Although widely used in clinical practice, a significant number of patients with CIDP do not respond to corticosteroids [11]. Moreover, long-term administration of corticosteroids is associated with numerous and potentially severe side effects. IVIG proved to be safe and potent in several RCTs in treatment-naïve patients as well as in steroid- or plasma-exchange nonresponders [10, 12–16]. Of 53 patients included in a short-term RCT [16], 30 were treated with IVIG and 23 with placebo, the latter being

clearly inferior. Hughes et al. [15] studied the effect of IVIG (2 g/kg over 1 to 2 days) vs. steroids (60 mg/day tapered to 10 mg/day) in a 6-week RCT crossover design. The patients clearly improved in both treatment arms. Although no significant difference could be detected between both treatment regimens, a trend toward a better response of IVIG was observed.

A recent clinical trial addressed short-term as well as long-term effects of caprylate/chromatography purified IVIG (IGIV-C) in comparison with placebo in CIDP [10]. Patients received placebo or a loading dose of 2 g/kg over 2 to 4 consecutive days, followed by regular infusions of 1 g/kg over 1 to 2 days every 3 weeks for up to 24 weeks. The primary outcome was the percentage of patients who had maintained an improvement from baseline to week 24. Patients who showed an improvement and completed 24 weeks of treatment were eligible to be randomly reassigned in a blinded 24-week extension phase.

During the first period, 32 of 59 (54%) patients treated with IGIV-C and 12 of 58 (21%) patients who received placebo had an improvement according to a clinical disability score (INCAT) that was maintained to week 24 (treatment difference 33.5%; 95% CI, 15.4–51.7; $P=0.0002$). During the extension phase, the participants who continued to receive IGIV-C had a longer time to relapse than the patients who were treated with placebo. This indicated prolonged benefits of maintenance treatment with IVIG in those patients with CIDP who initially responded to an IVIG loading dose. Thus, several prospective placebo-controlled clinical trials have demonstrated consistently that administration of IVIG improves neurologic disability and provides long-term benefits to patients with CIDP.

Anti-inflammatory Effects of IVIG: Fc is the Key

An IVIG preparation consists of the pooled serum IgG fraction from thousands of donors. Many mechanisms have been proposed to be involved in the anti-inflammatory activity of this therapeutic agent. Broadly speaking, these can be divided into two major pathways: either dependent on the F(ab)₂ or the Fc fragment of the IgG molecule. CD95-specific antibodies, for example, have been reported to be present in IVIG preparations and might interfere with the proapoptotic pathways essential for the pathology involved in a variety of skin-blistering diseases such as toxic epidermal necrolysis [17].

For most cases where suitable *in vivo* model systems were available, however, there is clear evidence that the IgG Fc fragment is the predominant mediator of the anti-

inflammatory activity. Thus, in animal models of immune thrombocytopenic purpura (ITP), rheumatoid arthritis, and nephritis, the Fc fragment but not the F(ab)₂ fragment interfered with autoantibody-triggered inflammation [18–20]. Similarly, the isolated IVIG Fc fragment prevented platelet depletion in human ITP patients, thus supporting the general importance of this Fc-dependent anti-inflammatory pathway [21].

Immunoglobulin G antibodies can trigger two major pro-inflammatory pathways via their Fc fragment. First, they can initiate activation of the complement pathway, which results in the generation of the proinflammatory anaphylatoxins C3a and C5a, and the lytic membrane attack complexes [22]. Second, IgG autoantibodies can cross-link cellular Fc receptors specific for IgG (FcγRs) that are present on most innate immune effector cells, including neutrophils, mast cells, and macrophages.

The family of FcγRs consists of several activating members (FcγRIA, IIA, IIC, and IIIA in humans; FcγRI, III, and IV in mice) and one inhibitory member (FcγRIIB), which are coexpressed on most innate immune effector cells. Thus, immune complexes will trigger both activating and inhibitory signalling pathways. Depending on the affinity of the different IgG subclasses for the inhibitory and the activating FcγRs, a more or less pronounced response will be initiated [23].

In addition to cells of the innate immune system, the inhibitory FcγRIIB is also expressed on B cells in which it transduces an inhibitory signal upon colligation with the B cell receptor. This action prevents B cells with low affinity or self-reactive receptors from entering the germinal center and becoming IgG-positive plasma cells [24]. Mice lacking FcγRIIB expression spontaneously develop autoimmune disease [25], and restoration of decreased FcγRIIB expression on activated B cells in autoimmune-susceptible mice restores immunological tolerance [24]. Autoimmune-prone mouse strains such as BXSB, NOD, and NZM carry a promoter polymorphism in the FcγRIIB gene that results in decreased protein expression [26]. Decreased FcγRIIB expression or nonfunctional FcγRIIB variants have been shown to be associated with the development and severity of systemic lupus erythematosus in several human populations [24, 27]. Notably, this inhibitory receptor is required for anti-inflammatory activity of IVIG because disruption of this protein by genetic deletion or via blocking antibodies reverses the therapeutic effects of IVIG in a variety of autoimmune animal models [19, 28–30].

Taken together, the inhibitory Fc-gamma receptor FcγRIIB plays a critical role in the balance of tolerance and autoimmunity and is required for the anti-inflammatory activity of IVIG in a variety of murine disease models. Less well understood, however, is the function of FcγRIIB and its regulation by IVIG in human autoimmune diseases.

Mechanisms of IVIG in CIDP

To address the function of FcγRIIB in an antibody-mediated IVIG-responsive human disease, we determined the expression profile of FcγRIIB on peripheral blood monocytes and B cells and explored its regulation following IVIG therapy as a possible pathomechanism in patients with CIDP [31].

We found that untreated patients with CIDP, compared with demographically matched healthy controls, showed consistently lower B cell expression levels of FcγRIIB. The reduction in FcγRIIB expression was stronger in the CD19⁺CD27⁺ memory compared with the CD19⁺CD27[−] naive B cell compartment. This was due to a failure of patients with CIDP to upregulate or to maintain upregulation of FcγRIIB as B cells became memory cells. FcγRIIB expression was unchanged in CD14⁺ blood myeloid cells. Next, we determined FcγRIIB expression levels on circulating monocytes and B cells in treatment-naïve patients with CIDP before and 2 to 3 weeks following clinically effective IVIG administration (2 g/kg body weight over 5 days). Compared with baseline levels, IVIG led to a significant upregulation of FcγRIIB expression on naive B cells, memory B cells, and to a lesser degree, blood monocytes in most of the patients. This indicates that the impaired expression of the inhibitory FcγRIIB in CIDP can, at least partially, be restored by clinically effective IVIG treatment.

Furthermore, these findings suggest that results obtained in murine model systems with respect to the mechanism of IVIG activity in vivo might be transferable to humans. These data also suggest that FcγRIIB expression mediates immunomodulatory effects of IVIG in CIDP by raising the activation threshold for B lineage and myeloid cells.

Outlook

New data from mouse models of rheumatoid arthritis suggest that certain IgG glycosylation variants might be of great importance for FcγRIIB binding and the anti-inflammatory activity of IVIG. Glycosylation of IgG-Fc contributes both to the stability and biological activity of antibody molecules and is essential for effector functions mediated through FcR. IgG and other immunoglobulin isotypes are composed of an amino acid backbone that contains a sugar moiety attached to an asparagine 297 residue in the antibody constant region (N297) [32]. This moiety consists of a heptameric core sugar structure with variable amounts of branching and terminal sugar residues such as galactose, sialic acid (SA), *N*-acetylglucosamine, and fucose. The importance of IgG glycosylation is highlighted by the loss of therapeutic activity of deglycosylated IVIG preparations [30]. Conversely, IVIG preparations and

isolated Fc fragments enriched for terminal SA residues have more than a tenfold higher anti-inflammatory activity [33].

More than 30 different antibody glycovariants have been detected in human serum, with about 25% of them located in the IgG-G0 glycoform, that is, without terminal SA or galactose residues. Notably, IgG-G0 glycoforms are markedly increased in patients with rheumatoid arthritis [34]. Especially during acute disease phases, a significant reduction in terminal SA residues in serum and antigen-specific antibodies can be observed, and the IgG-G0 glycovariant constitutes more than 50% of serum IgG [34]. Similar observations were made in experimental models of other autoimmune diseases [30, 35–39].

Thus, investigating Fc glycosylation in patients with CIDP as well as in other autoimmune diseases with a strong humoral immune component could provide new insights into mechanisms of antibody-mediated tissue damage. Although there are still many open questions regarding the mechanisms of this novel pathway, clinical trials to test the safety and therapeutic efficacy of SA-rich IVIG are currently being initiated. These trials might provide important information about the function of IgG glycosylation in the human immune system.

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